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TRANSLATIONAL RESEARCH

MitoVitE, a mitochondria-targeted antioxidant, limits paclitaxel-induced oxidative stress and mitochondrial damage *in vitro*, and paclitaxel-induced mechanical hypersensitivity in a rat pain model

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Abstract

Background: Neuropathic pain is a common side-effect of chemotherapy. Although precise mechanisms are unclear, oxidative stress and mitochondrial damage are involved. We investigated whether the mitochondria targeted antioxidant, MitoVitE, provided better protection against paclitaxel-induced mitochondrial damage in rat dorsal root ganglion (DRG) cells, than a non-targeted form of vitamin E, Trolox. We also determined whether MitoVitE, compared with duloxetine, could limit paclitaxel-induced mechanical hypersensitivity in rats.

Methods: Mitochondrial function was measured in DRG cells exposed to paclitaxel with and without MitoVitE or Trolox. The effect of MitoVitE or Trolox on paclitaxel-induced cell killing in cancer cell lines was also determined. Rats received a cumulative dose of 8 mg kg⁻¹ paclitaxel plus either MitoVitE (2 mg⁻¹ kg day⁻¹), duloxetine (10 mg kg⁻¹ day⁻¹) or vehicle control daily. Mechanical hind paw withdrawal thresholds were measured every two days.

Results: Paclitaxel caused loss of membrane potential in DRG cells. At 100 μM paclitaxel median [range] change was 61[44–78]%, $P < 0.0001$, which was ameliorated by MitoVitE (86[62–104]%) but not Trolox (46[46–57]%). Similarly, loss of metabolic activity and glutathione induced by paclitaxel (both $P < 0.0001$) were reduced by MitoVitE but not Trolox. Cytotoxicity of paclitaxel was not affected by co-exposure of ovarian cancer cells to either MitoVitE or Trolox, but was slightly reduced against breast cancer cells, in the presence of Trolox. Mean (SD) areas under the curve of withdrawal thresholds at 6 h after injection in rats given paclitaxel + control, or + MitoVitE ($P < 0.0001$) or + duloxetine ($P < 0.0001$) were 110 (5), 145 (10) and 156 (13) respectively.

Conclusions: Paclitaxel affected mitochondrial function and glutathione in DRG cells, which was abrogated by MitoVitE but not Trolox, without decreasing cancer cell cytotoxicity. In rats, paclitaxel-induced mechanical hypersensitivity was ameliorated by MitoVitE treatment to an extent similar to duloxetine. These data confirm mitochondria as a mechanistic target for paclitaxel-induced damage and suggest mitochondria targeted antioxidants as future therapeutic strategies.

Key words: mitochondria; Paclitaxel; pain

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Editor's key points

- Chemotherapy-induced peripheral neuropathy (CIPN) is a severe side-effect that frequently limits effective anti-cancer therapy.
- The mitochondria-targeted antioxidant MitoVitE, reduced oxidative toxicity of paclitaxel in a cultured sensory neuronal cell line, and reduced mechanical hypersensitivity in a rat model of CIPN.
- Mitochondrial oxidative toxicity contributes to CIPN; mitochondria-targeted antioxidants provide a novel mechanism-based preventative therapy.

Chemotherapy-induced peripheral neuropathy (CIPN) is a common and severe adverse effect of some commonly used chemotherapeutic drugs, including paclitaxel. The severity of symptoms can require a dose reduction, or even cessation of chemotherapy, impacting on survival. CIPN affects around 33% of patients, and can persist months or years beyond the cessation of treatment. It is characterized by numbness, paresthesia, and pain.^{1,2} Duloxetine is the only treatment that has been shown in a randomized clinical trial to be effective in some patients with CIPN,³ and is recommended as a first line treatment for adults with this condition. However, long term management of CIPN is often inadequate and there is urgent need for a mechanism-derived novel treatment.⁴

Mitochondria produce most of the body's cellular energy via oxidative phosphorylation, producing reactive oxygen species (ROS) in the process. Although ROS are potentially toxic, they have essential roles in cell signalling and any damage is controlled by an interacting and highly regulated system of endogenous antioxidants. In addition to being the main source of ROS, mitochondria are also a target for damage. When antioxidant defences are overwhelmed, oxidative stress can result in mitochondrial dysfunction and impairment of ATP production. Thus damage to mitochondria is caused primarily by ROS produced by the mitochondria themselves.⁵

Paclitaxel can cause oxidative stress and mitochondrial damage^{6,7} and accumulates in neuronal tissue. Neuronal cells are particularly sensitive to oxidative insults, and ROS have been implicated in many neurodegenerative processes, including Alzheimer's, Parkinson's, and Huntington's diseases, acute brain ischaemia, and excitotoxicity. Neuropathic pain as a result of paclitaxel therapy might be related to mitochondrial damage to neuronal cells,^{8,9} and so antioxidants that are able to protect mitochondria could be useful therapies. Antioxidants can be targeted selectively to mitochondria by conjugation to a lipophilic cation.¹⁰ MitoVitE consists of α -tocopherol attached to the triphenylphosphonium (TPP) cation, enabling its rapid uptake through the plasma and mitochondrial membranes and accumulation within mitochondria, as a result of the large membrane potential (negative inside) across the mitochondrial inner membrane. MitoVitE accumulates in all major organs of mice and rats after oral, intraperitoneal (i.p.) or i.v. administration.¹¹ Trolox (6-hydroxy-2,5,7,8-tetra methylchroman-2-carboxylic acid) is a synthetic, water soluble cell-permeable derivative of vitamin E, that accumulates in the cytosol and has potent antioxidant activity with direct scavenging activity against peroxy and alkoxy radicals.¹²

A number of rodent models of CIPN have been developed, including a paclitaxel-induced CIPN model. Neuropathic pain

develops over time, as a result of underlying neurobiological changes that includes mitochondrial dysfunction.¹³

The aim of this study was to investigate the effects of two vitamin E-based antioxidants in an *in vitro* model of paclitaxel-induced oxidative stress and mitochondrial damage in a (DRG) cell line, and effects on mechanical hypersensitivity in a pre-clinical rat model of paclitaxel CIPN.

Methods

In vitro studies

An immortalized rat DRG neuronal stem cell line (50B11) with nociceptive properties was used (a kind gift from Professor Ahmet Hoke, from Johns Hopkins School of Medicine, Baltimore, MA, USA). When differentiated, these cells extend neurites, express the capsaicin receptor transient receptor potential vanilloid family-1 (TRPV-1) and other receptors characteristic of small sensory neurones, generate action potentials when depolarized, and respond to capsaicin.¹⁴ Cells (used at passage 5–15) were grown to 70% confluence, in neurobasal media devoid of phenol red (Invitrogen, Paisley, UK), supplemented with 10% v/v foetal calf serum, B27 (Invitrogen, Paisley, UK), 2% w/v glucose, 0.5 mM L-glutamine, 50 μ g/ml gentamycin, 250 μ g/ml amphotericin-B and 5 μ g/ml ciprofloxacin, in a humidified incubator containing 5% CO₂ at 37°C. Cells were then treated with 75 μ M forskolin and allowed to differentiate into DRG for 24 h. Neurite outgrowth normally started around 10 h after addition of forskolin. After 24 h exposure to forskolin, paclitaxel (0–100 μ M) was added, corresponding broadly to concentrations seen in the circulation during clinical use. However, paclitaxel accumulates in cells and tissues and so the concentration range was extended to allow for this. In addition some cells were also concurrently exposed to 1 μ M MitoVitE, 1 μ M Trolox or solvent control.

Acid phosphatase activity

Acid phosphatase activity was used to assess cell viability.¹⁵ Differentiated cells were grown in 96-well plates and treated as described above for 24 h, then washed twice with phosphate buffered saline (PBS). Acid phosphatase solution containing 0.1M sodium acetate, 1% v/v Triton X-100, 5 mM p-nitrophenyl in distilled water (pH 5.0), was added to each well and cells were incubated in the dark for 1 h at 37°C. Sodium hydroxide (0.25 M) was added to stop the reaction and the absorbance measured using a spectrophotometer at 450 nm.

Mitochondrial function

Mitochondrial membrane potential was analyzed in intact cells using the fluorescent probe JC-1 (5,5,6,6-tetrachloro-1,1,3,3-tetraethylbenzimidazolcarbocyanine iodide, Invitrogen, Paisley, UK). Briefly, after 24 h treatments as described above, cells were washed with PBS and then incubated for 30 min with 7.5 μ M JC-1 in PBS at 37°C, in the dark. After incubation, cells were washed twice with PBS and the red/green fluorescence ratio was measured immediately.¹⁶ Results were corrected for cell viability.

Metabolic activity was assessed by measuring the rate of reduction of AlamarBlue™ in intact cells after 24 h treatment as described above.¹⁷ AlamarBlue™ (Invitrogen) is a redox indicator that exhibits both fluorescent and colourimetric changes, in response to changes in metabolic activity via oxidative metabolism. Briefly, after cell treatments, AlamarBlue™ was added to each well and fluorescence was measured every 15 min for 2 h

at 37°C. Metabolic activity was determined as the rate of change in fluorescence over time. Results were corrected for cell viability.

Glutathione measurement

Cellular glutathione (GSH) concentrations were measured as an indicator of oxidative stress. The lipophilic compound monochlorobromane (MCB, Sigma, Dorset, UK), binds to GSH via the action of the enzyme glutathione-S-transferase. The fluorescence of the resulting MCB-GSH conjugate is proportional to cellular GSH concentration.¹⁸ Cells were treated for 24 h as above, washed with PBS and incubated with 20 µM MCB for 30 min at 37°C. GSH was analysed by measuring fluorescence at excitation/emission wavelengths of 340/520 nm. Results were corrected for cell viability.

Cancer cell cytotoxicity

As it is possible that antioxidants would either interfere with or enhance the ability of paclitaxel to kill cancer cells, we also conducted experiments using the breast adenocarcinoma-like oestrogen-sensitive cell line, MCF-7, and the ovarian carcinoma cell line A2780, to assess paclitaxel-induced cell killing in the presence of antioxidants. Cells were cultured in Dulbecco's modification of Eagle's medium (DMEM) supplemented with 4.5 g/l glucose, 10% v/v foetal calf serum, 50 µg/ml gentamycin and 250 µg/ml amphotericin-B for 24 h. Cell viability was measured using acid phosphatase activity as described above.

Animal model

All studies were carried out in accordance with Animals (Scientific Procedures) Act 1986, and within the confines of project and personal licences issued by the UK Home Office, following relevant aspects of the ARRIVE Guidelines. Male Sprague Dawley rats, bred in-house and weighing 250–300 g (approximately seven weeks old) were used. Rats were housed up to six per cage at 19–22°C, on a 12-h light/dark cycle from 7am to 7pm, and had free access to food and drinking water. Animals were housed in the testing room throughout experiments and for at least three days before baseline sensitivity measures.

Rats were allocated by cage to receive either paclitaxel or vehicle control (Cremophor/EL and ethanol 1:1 [v/v], and diluted with saline as for paclitaxel; hereafter referred to as Cremophor) with and without MitoVitE or duloxetine as positive comparator. As treatment with paclitaxel can contaminate other animals via coprophagy, animals could not be individually randomized to treatment group. Rats received four doses of 2 mg kg⁻¹ paclitaxel or Cremophor by i.p. injection every second day,¹⁹ and also received either 2 mg kg⁻¹ day⁻¹ MitoVitE, 10 mg kg⁻¹ day⁻¹ duloxetine or equivalent vehicle control by daily i.p. injection, starting 7 d before paclitaxel administration, to allow steady-state tissue concentrations to be reached and to allow assessment of any effects in naïve animals. The injection site was varied daily to minimise local tissue damage.

For behavioural sensory testing, rats were acclimatised to testing apparatus in 2 x 20 min sessions on separate days before testing, and further habituated for 20 min immediately before testing on any given day. To ensure blinding of the tester to the treatment, rats from several cages were briefly combined in a single cage before testing commenced; group allocation was only confirmed by tail number after testing was complete. Mechanical withdrawal thresholds were measured using the up-down method using a series of von Frey filaments of varying

weights, to gauge the withdrawal threshold and thereby mechanical sensitivity.²⁰ Starting with a 2 g filament, increasingly heavier filaments were applied to the plantar region of the hind paw, until a paw withdrawal response was observed. Sequentially smaller filaments were then tested until no response was observed, then increased again until a response was observed. This approach was repeated until there were five measures after the initial response. The value of the final filament tested, and sequence of responses were then used to calculate the mechanical threshold, using the equation devised by Chaplan and colleagues.²⁰ Behavioural measures were undertaken three, six and nine h after the MitoVitE or duloxetine injection, every two d starting three d before paclitaxel administration and continuing until 14 d after initial paclitaxel administration.

Statistical analysis

For *in vitro* studies, six independent experiments with four technical replicates were undertaken (n = 6). No assumptions were made about data distribution and all data are presented as median, interquartile/full range. Statistical analysis was undertaken using Analyse-it add in for Microsoft Excel (Analyse-it Software Ltd., Leeds, UK). Kruskal Wallis analysis of variance was used for each *in vitro* treatment, with Mann-Whitney *U*-test *post hoc* testing and correction for multiple comparisons as appropriate. For *in vivo* studies five to nine rats per group were used. Data are presented as mean (SD) and were analysed using two-way repeated measures ANOVA with Bonferroni-corrected *post hoc* comparisons (Graph-Pad Prism v5.0, California, USA). A *P* value <0.05 was considered to be significant.

Results

In vitro studies

Acid phosphatase activity

There was a significant concentration-dependent effect of paclitaxel on DRG cell viability, such that at 100 µM paclitaxel the median [range] viability was 66[58–88]%. However there was no additional effect of MitoVitE or Trolox (see [Supplementary data](#)). All subsequent measures were corrected for median viable cell number.

Mitochondrial function

Exposure of DRG cells to paclitaxel at all doses without antioxidants resulted in decreased JC-1 red/green fluorescence ratio, indicating a loss of mitochondrial membrane potential ($P < 0.0001$, [Fig. 1A](#)). Mitochondrial membrane potential in cells without paclitaxel was similar to cells with antioxidant only, but in cells co-treated with paclitaxel and MitoVitE, membrane potential was only decreased at the highest dose of paclitaxel ([Fig. 1A](#)). Cells treated with Trolox had similar loss of membrane potential to paclitaxel alone ([Fig. 1A](#)), suggesting that it was ineffective at preventing loss of potential induced by paclitaxel. Mitochondrial metabolic activity was significantly lower in cells treated with paclitaxel, regardless of dose ($P < 0.0001$, [Fig. 1B](#)). In cells co-treated with MitoVitE, there was no decrease in metabolic activity even at the highest paclitaxel dose ([Fig. 1B](#)). Trolox in contrast, worsened the loss of metabolic activity ([Fig. 1B](#)).

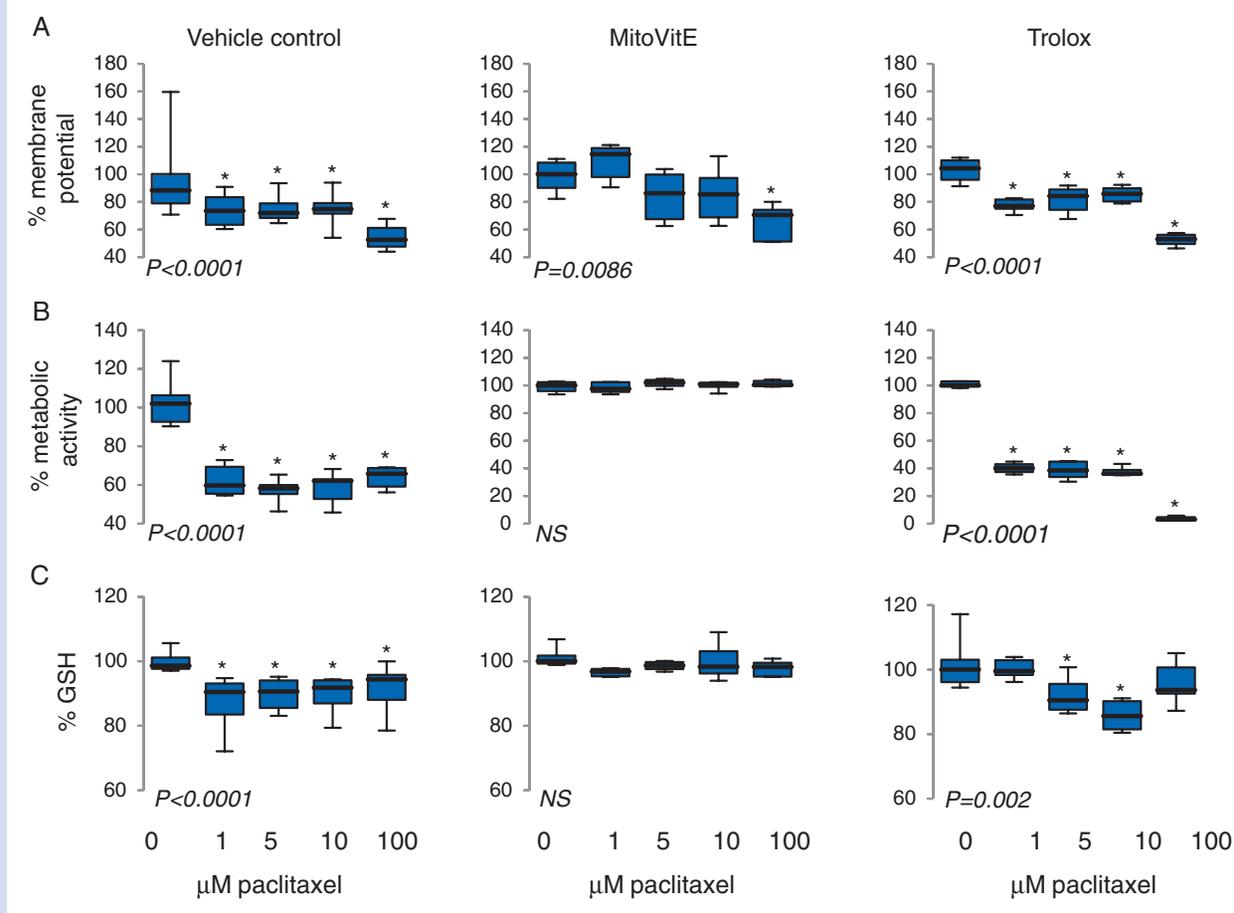


Fig 1 Effect of a range of concentrations of paclitaxel plus 1 μM MitoVitE or Trolox, or vehicle control on (A) mitochondrial membrane potential, (B) mitochondrial metabolic activity and (C) glutathione (GSH) in dorsal root ganglion cells. Results are presented as percentage of data at baseline (i.e. vehicle control treated cells without paclitaxel but with antioxidant treatment), to facilitate comparisons between treatments. Data are shown as box and whisker plots showing median, interquartile and full range ($n=6$). P value is Kruskal Wallis. * = significantly different to without paclitaxel.

Glutathione concentrations

Compared with control treatments, cellular GSH concentrations were significantly lower in DRG cells exposed to paclitaxel without antioxidant, independent of paclitaxel concentration ($P < 0.0001$, Fig. 1C). Co-exposure of paclitaxel with MitoVitE but not Trolox, mitigated the effect on GSH (Fig. 1C).

Cancer cell cytotoxicity

Exposure of the A2780 ovarian carcinoma cell line, or the MCF7 breast adenoma cell line to paclitaxel, resulted in concentration-dependent decreases in acid phosphatase activity, indicating loss of cell viability, such that at 100 μM paclitaxel, only 60% of cells were viable. Co-exposure of cells to paclitaxel plus MitoVitE showed similar loss of viability (Figs 2A and 2B). Similar results were seen in Trolox treated ovarian cancer cells (Fig. 2A), but paclitaxel mediated killing of breast cancer cells was less effective in the presence of Trolox (Fig. 2B).

As a result of the promising effects of MitoVitE observed *in vitro* we then went on to assess its effects *in vivo*, with duloxetine as comparator.

In vivo studies

None of the treatments affected weight gain (Supplementary file). There was also no effect of MitoVitE or duloxetine on withdrawal thresholds in the absence of paclitaxel administration (i.e. in naïve animals) (Supplementary file).

Mechanical withdrawal thresholds of a hind paw were examined, every second day, at three, six and nine h after the daily injection of MitoVitE or duloxetine (Fig. 3). Rats receiving paclitaxel with vehicle control treatments, had significantly lower withdrawal threshold values, compared with those receiving Cremophor from four days after the first paclitaxel injection, until the end of the study (day 14). Rats receiving paclitaxel plus duloxetine had significantly higher withdrawal threshold values, than rats given paclitaxel plus vehicle control on days eight-12. However rats receiving paclitaxel plus MitoVitE had significantly higher withdrawal threshold values than rats given paclitaxel plus vehicle control, over the longer timeframe of days four-14 (with the exception of day six).

For both MitoVitE and duloxetine treatment groups, significantly increased withdrawal thresholds were not consistent across the three, six and nine h time points, indicating time-

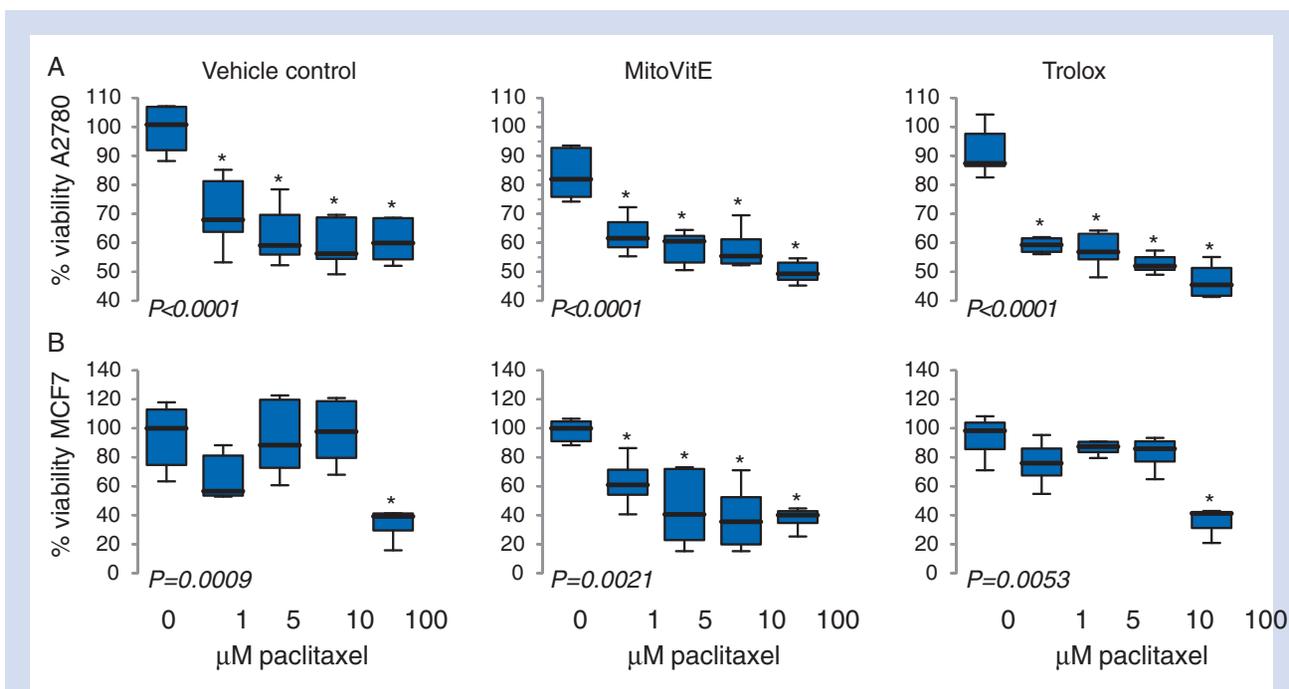


Fig 2 Effect of a range of concentrations of paclitaxel plus 1 μ M MitoVitE or Trolox, or vehicle control on cell viability in (A) the A2780 ovarian carcinoma cell line and (B) in the MCF-7 breast adenocarcinoma-like oestrogen-sensitive cell line. Results are presented as percentage of data at baseline (i.e. vehicle control treated cells without paclitaxel but with antioxidant treatment), to facilitate comparisons between treatments. Data are shown as box and whisker plots showing median, interquartile and full range ($n = 6$). P value is Kruskal Wallis. * = significantly different to without paclitaxel.

dependent drug effects (Fig. 3). To directly assess this, area under the curve (AUC) values between days 0 and 14 were calculated for each individual animal at each time point (Fig. 4A). There was no significant difference between three, six and nine h time points in the rats receiving either Cremophor or paclitaxel plus vehicle control. However in rats receiving paclitaxel plus duloxetine there was a significant effect of time, with a peak effect at six h. In contrast, although there was a significant effect of time in rats receiving paclitaxel plus MitoVitE, there was a progressively increasing effect between three and nine h. This led us to assess an additional 24 h time point after the last drug/vehicle administration (day 14, Fig. 4B). There was a significant effect at the six h time point, only in rats treated with paclitaxel plus duloxetine, compared with rats given paclitaxel plus vehicle control. The effect was more prolonged in rats receiving paclitaxel plus MitoVitE with a significant effect at six, nine and 24 h (Fig. 4B).

Discussion

We found that in DRG cells *in vitro*, paclitaxel affected aspects of mitochondrial function. There was loss of mitochondrial membrane potential, reduced metabolic activity, and loss of glutathione. Co-treatment of cells with paclitaxel plus MitoVitE, but not Trolox, protected against these effects. MitoVitE also did not decrease the ability of paclitaxel to kill cancer cells in two different cell lines, whilst cytotoxicity against breast cancer cells was decreased by Trolox. In a rat model of chemotherapy-induced neuropathic pain, we also showed that MitoVitE decreased paclitaxel-induced mechanical hypersensitivity, to a similar degree as duloxetine. Moreover, at the doses tested, the duration of action of MitoVitE was longer than that of duloxetine.

Chemotherapy remains the mainstay of cancer treatment for solid tumours, but side-effects can be severe enough to limit treatment. Paclitaxel acts by binding to microtubules and causing arrest of mitosis in cancer cells, followed by apoptotic cell death. Although proliferating cancer cells are susceptible to the action of paclitaxel, neuronal cells are also targets for damage. It has been shown that paclitaxel initiates opening of the mitochondrial permeability transition pore, leading to loss of mitochondrial function in DRG cells.²¹ In paclitaxel treated rats, neuropathic pain behaviour is associated with evidence of mitochondrial damage in peripheral nerves,⁹ and administration of mitochondrial poisons such as rotenone worsens such behaviours.²²

The main function of mitochondria is to produce energy via oxidative phosphorylation but they are also an important source of ROS production, essential for signalling pathways. Although excessive mitochondrial ROS can be detrimental, antioxidant systems work in synergy to control potential damage under normal conditions.⁵ Exposure of a variety of cell types to paclitaxel, results in increased ROS production and oxidative stress.^{7, 8} In animals, treatment with spin trap global radical scavengers, reduced pain behaviour induced by paclitaxel.²³ Recently, an inhibitor of mitochondrial p53 accumulation was reported to limit paclitaxel induced mitochondrial damage and prevented mechanical allodynia. These findings support the mechanistic importance of mitochondrial dysfunction and the rationale for targeting treatment specifically at mitochondria.²⁴

Antioxidants can be modified to specifically target mitochondria and increase efficacy. MitoVitE is able to enter mitochondria by virtue of the TPP cationic conjugate, which enables accumulation several hundred fold higher inside the mitochondrion

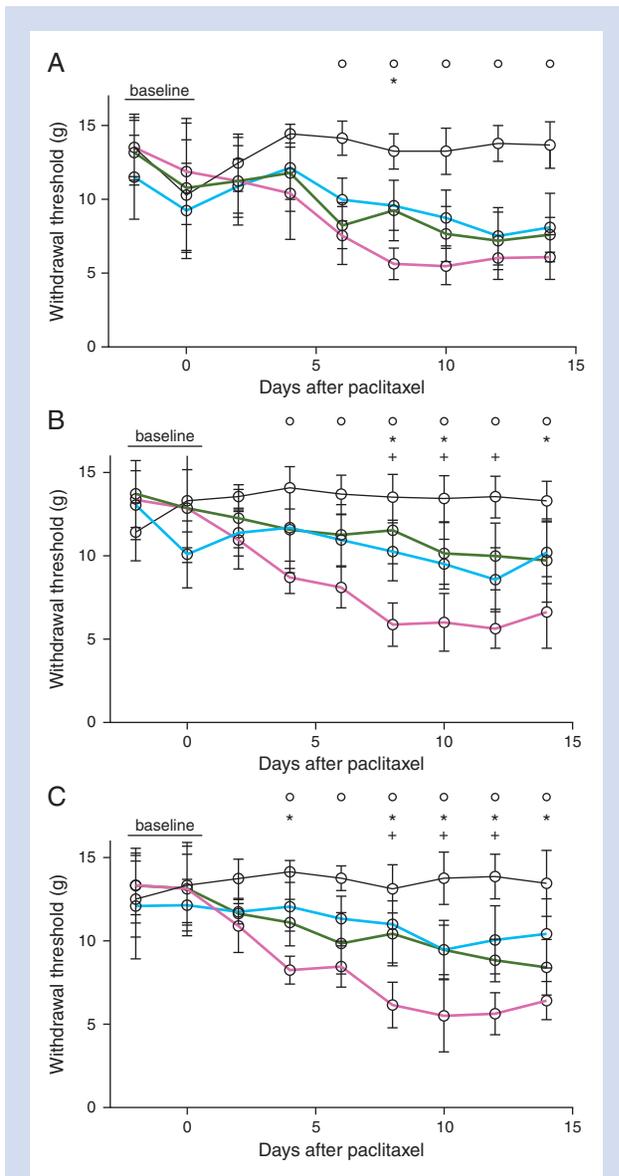


Fig 3 Mechanical hind paw withdrawal thresholds of groups receiving Cremophor (black), paclitaxel with vehicle control (red), paclitaxel with duloxetine (green) and paclitaxel with MitoVitE (blue). Mechanical withdrawal thresholds measured at (A) three h (B) six h and (C) nine h after drug or vehicle administration. Data are shown as mean (SD), n = 5–9 per treatment group. Two-Way ANOVA followed by Bonferroni post hoc test used to compare all groups to paclitaxel with vehicle control. o = Cremophor $P < 0.001$, * = paclitaxel with MitoVitE $P < 0.05$, + = paclitaxel with duloxetine $P < 0.05$.

relative to cytosolic levels, where it is many times more effective at protecting against oxidative damage to mitochondria, than other non-targeted antioxidants.^{10–11} We and others have shown that MitoVitE is effective in other disease models involving mitochondrial damage and oxidative stress.^{25–26} Trolox is a water-soluble derivative of α -tocopherol that permeates cells easily but cannot protect inside mitochondria. It is beneficial against oxidative stress in both cells and animals, but has far lower antioxidant activity than MitoVitE in standard *in vitro* rat brain homogenate lipid peroxidation assays.²⁷ In DRG cells *in vitro*, we

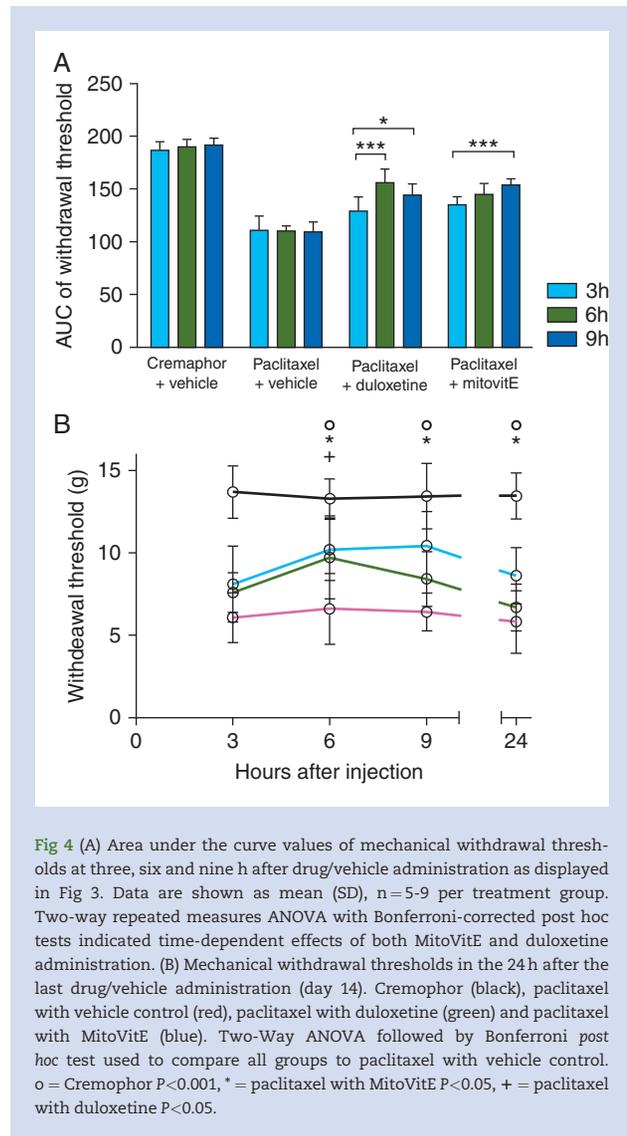


Fig 4 (A) Area under the curve values of mechanical withdrawal thresholds at three, six and nine h after drug/vehicle administration as displayed in Fig 3. Data are shown as mean (SD), n = 5–9 per treatment group. Two-way repeated measures ANOVA with Bonferroni-corrected post hoc tests indicated time-dependent effects of both MitoVitE and duloxetine administration. (B) Mechanical withdrawal thresholds in the 24 h after the last drug/vehicle administration (day 14). Cremophor (black), paclitaxel with vehicle control (red), paclitaxel with duloxetine (green) and paclitaxel with MitoVitE (blue). Two-Way ANOVA followed by Bonferroni post hoc test used to compare all groups to paclitaxel with vehicle control. o = Cremophor $P < 0.001$, * = paclitaxel with MitoVitE $P < 0.05$, + = paclitaxel with duloxetine $P < 0.05$.

found that MitoVitE, but not Trolox, protected against mitochondrial dysfunction induced by paclitaxel.

Mitochondrial membrane potential is determined by the efficiency of the electron transport chain which creates the membrane potential, and the inner membrane permeability.²⁸ Using a novel flow cytometry technique, Zhang and colleagues²⁹ reported that paclitaxel concentration, dose dependently reduced the membrane potential of isolated mitochondria from HeLa cells. JC-1 is a widely used tool for measurement of mitochondrial membrane potential and is thought to be a more reliable measure than other cationic probes,^{16–28} although it must be interpreted alongside other measures of mitochondrial function. In isolated mitochondria from human neuroblastoma, paclitaxel induces release of cytochrome C and loss of mitochondrial membrane potential,³⁰ and in human melanoma cell lines decreases expression of mitochondrial uncoupling protein 2 (UCP2), with increased ROS generation and loss of mitochondrial membrane potential suggesting opening of the mitochondrial pore.³¹ We also found that paclitaxel caused loss of mitochondrial membrane potential in intact DRG cells.

We used AlamarBlue as a global measure of mitochondrial metabolic activity.^{16 17} AlamarBlue can be reduced by NADPH, FADH, FMNH, NADH and the cytochromes, thus allowing the respiratory chain to function to near completion. We found that paclitaxel decreased metabolic activity, and that MitoVitE, but not Trolox, abrogated this. Decreased metabolic activity is probably an adaptive mechanism, as a result of increased UCP2 expression in response to increased ROS formation. UCP2 acts as a uncoupler to reduce ROS formation and not ATP formation as indicated by the degree of decline in the mitochondrial membrane potential. Decreased metabolic activity represents a protective response.

Increased total GSH can suggest oxidative stress, but consumption and loss of GSH can represent overwhelming of the GSH system. We found that GSH concentrations decreased in DRG cells exposed to paclitaxel, and this was less marked in cells treated with MitoVitE but not Trolox. In mouse tissue slices, paclitaxel-mediated release of calcitonin gene-related peptide, a sensory neuropeptide implicated in paclitaxel-induced neuropathy, was abolished by treatment with GSH.³²

Antioxidants protect against chemotherapy-induced oxidative stress and so addition of antioxidants to cancer chemotherapeutic regimens could in theory decrease their efficacy in killing cancer cells. The efficacy of cell killing in breast carcinoma cell lines by some chemotherapeutic drugs is reduced by concomitant treatment with non-biological antioxidants.³³ Although resveratrol, a naturally occurring antioxidant found in grapes and other red berries, reduced the efficacy of paclitaxel to kill some breast cancer cell lines, susceptibility of MCF-7 cells was not diminished.³⁴ However a subsequent study found increased cytotoxicity of paclitaxel in the presence of resveratrol even in paclitaxel resistant breast cancer cell lines.³⁵ However we found that MitoVitE did not inhibit the cytotoxic action of paclitaxel against two different cancer cell lines, whereas Trolox seemed to reduce killing in MCF7 cells. In attempts to improve drug delivery, vitamin E has been used to form D- α -tocopherol polyethylene glycol (PEG) 1000 succinate, by the esterification of tocopherol succinate with PEG 1000, to create a redox sensitive paclitaxel pro-drug which increased paclitaxel-induced cytotoxicity in A549 cells.³⁶

We used the well-characterized rat model of paclitaxel-induced neuropathic pain, where rats exhibit mechanical allodynia indicated by reduced mechanical withdrawal thresholds as assessed using Von Frey filaments.^{19 20} Several rat models of paclitaxel-induced CIPN exist, which use a wide range of doses, treatment regimens and routes of administration. The specific model used here is amongst the most widely used and best characterized of paclitaxel-induced CIPN models.¹⁹ We found that MitoVitE (a mitochondria targeted form of tocopherol), limited paclitaxel-induced mechanical hypersensitivity to a similar level as that seen with duloxetine, and moreover had a longer duration of action. Duloxetine has been shown to reduce neuropathic pain in clinical trials in patients treated with chemotherapy.³⁴ In rat models of neuropathy other than that induced by paclitaxel, tocopherol (naturally occurring lipid soluble vitamin E) reduces allodynia after sciatic nerve crush injury,³⁷ chronic constriction induced ischaemic injury³⁸ and oxaliplatin-induced neuropathy.³⁹ MitoVitE is more effective in reducing mitochondrial damage in rat models of sepsis, another condition involving oxidative stress,^{25 26} but there have been no studies describing its use in neuropathy or pain.

MitoVitE has not been through Phase I trials and so cannot currently be used in patients; however this study demonstrates proof of concept regarding a beneficial effect of mitochondria

targeted antioxidant protection for CIPN. Other antioxidants that specifically protect inside mitochondria might be suitable for clinical use.⁴⁰ Further work should assess different antioxidant doses, timing of administration in relation to pain onset, and potential synergy with duloxetine. It would also be of interest to study its impact on underlying neuropathology by quantifying intraepidermal nerve fibre density.⁴¹

In summary, we showed that paclitaxel-induced mitochondrial damage in DRG cells *in vitro*, and that this was ameliorated by MitoVitE but not Trolox. In a rat model of paclitaxel-induced neuropathic pain, MitoVitE was as effective at reducing mechanical hypersensitivity as duloxetine, the first line treatment for patients with paclitaxel-induced neuropathic pain. These results confirm the role of mitochondrial oxidative damage in paclitaxel-mediated CIPN and suggest novel treatment strategies.

Authors' contributions

Study design/planning: C.T., L.C., H.F.G.

Study conduct: B.M., C.T., D.L., L.C., H.F.G.

Data analysis: B.M., C.T., H.F.G.

Writing paper: C.T., D.L., L.C., H.F.G.

Revising paper: all authors

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Declaration of interest

H.F.G. and L.C. are Editors and members of the Board of Management of the *British Journal of Anaesthesia*.

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